Thermal adaptation occurs in the respiration and growth of widely distributed bacteria

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Abstract

Microbial thermal adaptation will lead to a weakening of the positive feedback between climate warming and soil respiration. The thermal adaptations of microbial communities and fungal species has been widely proven. However, studies on the thermal adaptation of bacterial species, the most important decomposers in the soil, are still lacking. Here, we isolated six species of widely distributed dominant bacteria and studied the effects of constant warming and temperature fluctuations on those species. The results showed that both scenarios caused a downregulation of respiratory temperature sensitivity (Q10) of the bacterial species, accompanied by an elevation of the minimum temperature (Tmin) required for growth, suggesting that both scenarios caused thermal adaptation in bacterial species. Fluctuating and increasing temperatures are considered an important component of future warming. Therefore, the inclusion of physiological responses of bacteria to these changes is essential the prediction of global soil-atmosphere C feedbacks.

Short running title: Warming causes thermal adaptation in bacteria

Thermal adaptation occurs in the respiration and growth of widely distributed bacteria

Keywords: bacteria, thermal adaptation, temperature fluctuation, Q_{10}, T_{min}

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AUTHORSHIP

M.N. developed the original ideas presented in the manuscript; W.T. completed the experiments with the assistance from Y.Z. and H.S.; W.T. performed the overall analysis with the assistance from H.S., J.Y. and J.X.; W.T., H.S., B.L. and M.N. wrote the first draft, and all authors jointly revised the manuscript.

DATA ACCESSIBILITY STATEMENT

The original data for this study will be publicly available at Zenodo.

ABSTRACT

Microbial thermal adaptation will lead to a weakening of the positive feedback between climate warming and soil respiration. The thermal adaptations of microbial communities and fungal species has been widely proven. However, studies on the thermal adaptation of bacterial species, the most important decomposers in the soil, are still lacking. Here, we isolated six species of widely distributed dominant bacteria and studied the effects of constant warming and temperature fluctuations on those species. The results showed that both scenarios caused a downregulation of respiratory temperature sensitivity (Q_{10}) of the bacterial species, accompanied by an elevation of the minimum temperature (T_{min}) required for growth, suggesting that both scenarios caused thermal adaptation in bacterial species. Fluctuating and increasing temperatures are considered an important component of future warming. Therefore, the inclusion of physiological responses of bacteria to these changes is essential the prediction of global soil-atmosphere C feedbacks.

Keywords: bacteria, thermal adaptation, temperature fluctuation, Q_{10}, T_{min}

INTRODUCTION

The microbial decomposition of soil organic matter (SOM) can produce up to 13.5 Gt CO_2 y^{-1}, which is comparable to the demand of terrestrial plants (Gruber & Galloway 2008; Ni *et al*. 2021). Bacterial species are abundant in soils, and these bacteria play a key role in soil carbon (C) cycling and nutrient exchange (Delgado-Baquerizo *et al*. 2018; Janssen 2006). Among soil bacteria and fungi, the biomass of soil bacteria is as high as 70%~90%, making them the most active biological factor in the soil (Bardgett & van der Putten 2014). Temperature is one of the most important factors regulating soil microbial biomass and respiration (Jonathan & Taylor 1994). Studies in recent years demonstrated that after initially being enhanced by warming in the short term, microbial community respiration would then be expected to continuously or periodically recover to previous levels—or even decrease—under long-term warming across diverse terrestrial ecosystems (Bárcenas-Moreno *et al*. 2009; Bradford *et al*. 2008, 2010; Wei *et al*. 2014). As the temperature sensitivity of respiration (Q_{10}) decreases (Crowther & Bradford 2013; Mahecha *et al*. 2010), thermal adaptation in microbial respiration is generated, which has been widely demonstrated in the soil layer (Bárcenas-Moreno *et al*. 2009; Birgander *et al*. 2018; Curiel Yuste *et al*. 2010; Luo *et al*. 2001; Wang *et al*. 2016). The main contributors to soil microbial respiration are fungi and soil bacteria, but the temperature sensitivity of these communities is different (Heinemeyer *et al*. 2012). For accurate prediction of the C release from soils in response to future warming, it is important to add microbial physiological responses into C cycle models (Allison *et al*. 2010; Conant *et al*. 2011; Reich 2010; Treseder *et al*. 2012). Previous studies have fully demonstrated the thermal adaptation of individual fungal and bacterial communities (Bradford *et al*. 2008; Crowther & Bradford 2013; Malcolm *et al*. 2008; van Gestel *et al*. 2020); therefore, it is well founded to speculate that the responses of individual species of bacteria to temperature would be

similar to that of the bacterial community, but studies of individual bacterial species are much less developed (Bennett & Lenski 2007; Rousk *et al*. 2012).

Temperature is the major force driving the growth and respiration of soil bacteria (Davidson & Janssens 2006). Constant warming-mediated thermal adaptation of bacteria is thought to originate from changes in community structure (Hartley *et al*. 2008; Wallenstein *et al*. 2007). That is, changes in the structures and compositions of microbial species communities and/or physiological modifications diminish the positive feedback of higher temperatures on bacterial respiration (Bárcenas-Moreno *et al*. 2009; Hartley *et al*. 2008; Wallenstein *et al*. 2007). However, it remains uncertain how individual bacterial species respond to constantly increasing temperatures. In addition, previous studies have focused on the effects of constant warming on thermal adaptation in microbial communities (Bennett & Lenski 2007; Crowther & Bradford 2013), but the temperature in the in situ environment usually fluctuates. Furthermore, according to the IPCC 6th Assessment Report, global changes in temperature fluctuate (IPCC 2021). It has been suggested that fluctuating temperature conditions during incubation can induce changes in the phenotypic plasticity of bacteria, such that evolution will lead to diverse genotypes (DeWitt & Scheiner 2004; Levins 1968), improving the ability of bacteria to withstand temperature fluctuations and maintaining the abundance and polymorphism of genetic variation (Levene 1953; Mackay 1980). In turn, temperature fluctuations lead to changes in the physiological responses of bacteria (e.g., reduced respiration, slower growth rates) (Biederbeck & Campbell 1973; Ketola & Saarinen 2015; Zhu & Cheng 2011). Thus, in addition to constant warming, fluctuating temperatures may also cause bacterial thermal adaptation. However, there is still a gap in studies on how temperature fluctuations affect the respiration of individual bacterial species. Thus, to better predict the potential contributions of soil bacteria to respiration, it is important to clarify the bacterial

responses to constantly increasing temperatures and temperature fluctuations.

Mass-specific respiration rates (R_{mass}) are generally applied to describe bacterial respiration per net unit biomass after eliminating biomass differences (Bradford *et al*. 2008; Tjoelker *et al*. 2008). Normalization of biomass is important because bacterial adaptation to temperature changes involves changes in mass-specific respiration rates and instantaneous temperature compensation (Hazel & Prosser 1974; Hochachka & Somero 2002; Tjoelker *et al*. 2008). The responses of bacteria contrast the temperature trends to which they are exposed; that is, R_{mass} decreases after a continuous increase in temperature, i.e., the Q_{10} decreases, and increases after a continuous decrease in temperature (Bradford *et al*. 2008; Dacal *et al*. 2019; Karhu *et al*. 2014). On the other hand, the lowest temperature at which bacteria starts to grow, i.e., the minimum temperature for growth (T_{min}), is a valid indicator of the temperature adaptation of bacterial growth (Li & Dickie 1987). Bacterial T_{min} is not dependent on the temperature range involved in an experiment and can be used to calculate changes in bacterial growth activity over any temperature interval (Bååth 2018; Nottingham *et al*. 2019). Typically, T_{min} is lower for bacteria adapted to low temperatures than for those adapted to high temperatures (Nottingham *et al*. 2019). The square root relationship model proposed by Ratkowsky *et al*. in 1983 (Ratkowsky *et al*. 1982, 1983) is considered an effective method for describing the growth rate of bacteria at different temperatures (Bååth 2018; Bradford *et al*. 2019; Gregson *et al*. 2020; Li *et al*. 2021). The model allows the direct effect of temperature on bacterial growth as well as the thermal adaptation of bacteria to be observed (Bååth 2018). T_{min} provides a direct estimate and prediction of the effect of climate warming on bacterial physiological activity (Nottingham *et al*. 2019; Ratkowsky *et al*. 1983). We therefore predict that after adaptation of individual bacterial species to higher or fluctuating temperatures, there will be a downregulation of bacterial R_{mass} and a decrease in Q_{10}, accompanied by an elevation in the

113 T_{min} of bacterial growth.

114

115 # MATERIAL AND METHODS

116 **Cultivation of soil bacteria**

117 The soil for the experiments was taken from a national forest park in Yichang, Hubei Province
118 (111'21"E, 30'48"N), where there is little human disturbance and relatively pristine vegetation and soil
119 environments remain. The average annual temperature of the sampling site is 17°C, and the maximum
120 daily temperature is 34°C. The average daily temperature fluctuations were 8°C, and the maximum
121 daily temperature fluctuations were 27°C. After isolating and culturing the soil bacteria, the most
122 dominant species were selected for the experiment. These six widely distributed species (i.e.,
123 *Chlororaphis* sp., *Pauculus* sp. (phylum Proteobacteria), *Xylanilyticus* sp., *Proteolyticus* sp.,
124 *Megaterium* sp. and *Wiedmannii* sp. (phylum Firmicutes)) are the predominant bacteria in soils
125 worldwide and are highly represented and similarly distributed globally (Delgado-Baquerizo *et al*.
126 2018). The bacterial 16S DNA sequences of these species were identified by EzBioCloud. The bacteria
127 were incubated at four temperature settings in lysogeny broth (LB) culture medium. The control group
128 was incubated at a constant temperature of 20°C (simulating the average annual temperature of the in
129 situ soil). The experimental groups were incubated at 20 ± 5°C (10°C temperature fluctuations), 20 ±
130 15°C (30°C temperature fluctuations) and 35°C (constant warming; the maximum temperature of the
131 soil in situ during the growing season). In particular, the temperatures were fluctuated once for a period
132 of 24 hours, during which the average temperature of both variable temperature groups was 20°C. Each
133 species was cultured in these four temperature regimes for more than 10 generations (over a period of

15 days) to ensure adequate observation of the temperature response of the bacteria after sufficient subculturing (Bradford *et al*. 2008; Hochachka & Somero 2002).

Respirations and Q_{10}

The bacteria were transferred to liquid LB medium upon completion of the secondary culture and cultured in respiration flasks with sealed rubber stoppers and three-way valves. The temperatures at which respiration was tested were 5°C, 15°C, 25°C and 35°C. After the bacteria were cultured at the test temperatures for approximately 18 hours, the air over the bottles was replaced with pure CO_2-free air (the initial CO_2 concentration was 0). The bacteria were then cultured at the test temperatures for one additional hour, and then the incubations were finished. The air over the bottles was extracted, and the CO_2 concentrations were measured by gas chromatography (Agilent 6890; Agilent Corp, USA), while the concentrations of the bacteria in the bottles were measured by an enzyme-labeled instrument (Synergy™ HTX, BioTek, USA). The mass-specific respiration rates (R_{mass}) were calculated as:

$$R_{mass} = R / (m \cdot V)$$

where R is the variation in the concentration of CO_2 (Δppm) measured in equal volume over the respiration bottle, m is the concentration of the bacterial liquid measured in the bottles at 600 nm wavelength, and V is the total volume of the bacterial liquid in the bottles (L).

After obtaining the R_{mass} of the bacteria, the respiratory temperature sensitivity (Q_{10}) was calculated:

$$Q_{10} = (R_{mass2}/R_{mass1})^{\left(\frac{10}{T_2-T_1}\right)}$$

where R_{mass1} and R_{mass2} are the per unit bacterial respiration rates measured at temperatures T_1 and T_2 (°C), respectively (where $T_1 < T_2$), with identical units of R_{mass1} and R_{mass2}. The temperature gap

between T_1 and T_2 was not required to be 10°C.

Bacterial growth and T_{min}

To fit the curve of bacterial growth with respect to temperature, bacteria cultured entirely at the incubation temperatures were transferred to liquid LB medium, and each species was grown at seven test temperatures (10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C). Biomass was sampled in 96-well plates to estimate the optical density at 600 nm. The specific turbidity of each species in the stationary phase was recorded in advance as a 100% value, and the time taken to reach 35% specific turbidity for each bacteria at each test temperature was recorded to calculate T_{min} according to the Ratkowsky square root relationship (Ratkowsky *et al*. 1983):

$$\sqrt{r} = b\,(T - T_{min}) \quad (1)$$

where r is the growth rate constant of the bacteria, expressed in the experiment as the reciprocal of the time taken for the bacteria to reach 35% turbidity, b is the regression coefficient, and T is the test temperature. However, when the temperature exceeds the optimum temperature for bacterial growth, the bacterial activity decreases, accompanied by a decrease in the growth curve due to, for example, changes in protein structure. Hence, the equation is extended to describe the relationship more completely (Ratkowsky *et al*. 1983):

$$\sqrt{r} = b\,(T - T_{min})\,\{1 - \exp\,[c\,(T - T_{max})]\} \quad (2)$$

where T_{min} and T_{max} are the minimum and maximum temperatures for bacterial growth, respectively, i.e., bacteria stop growing when the temperatures reach T_{min} and T_{max}, b is a parameter in equation (1), and c is an additional parameter. When T is much lower than T_{max}, $\sqrt{r}$ is linear with T, allowing a more accurate estimation of bacterial T_{min}.

Data analysis

One-way ANOVA was conducted to analyze the differences in the bacterial R_{mass} and Q_{10} in the treatments. Based on equations (1) and (2), square root relationship curves were plotted, and the coordinates of the intersections of the curves with the x-axis, i.e., the bacterial T_{min}, were calculated. The trend of T_{min} was statistically analyzed using a paired t-test with bacterial species as replicates. The t-test and the paired t-test were conducted with the R packages t.test and stats, respectively (v 3.6.1, R Core team, 2019). The curves were fitted in Origin (v 2019b).

RESULTS

Bacterial R_{mass} and Q_{10}

The respiration curves of the six bacterial species were fitted using an exponential model ($p < 0.001$ for all species). After 10 generations at the incubation temperatures, the experimental groups exposed to 15°C warming, 10°C temperature fluctuations and 30°C temperature fluctuations exhibited a downturn in bacterial R_{mass} compared to that of the group exposed to the control temperature (20°C), reflecting the rapid adaptation of the bacteria to temperature in approximately 15 days. At high test temperatures, the respiration of the bacteria adapted to the higher incubation temperatures decreased compared to that of the control group ($p < 0.05$ for all bacteria at a test temperature of 35°C, Fig. 1), while at lower test temperatures, the respiration of the control group was similar to that of the experimental group.

To further support these findings, we calculated the respiratory Q_{10} of the bacteria at all the test temperatures, and the results showed a significant decrease in Q_{10} for all the experimental groups

196 (warming & temperature fluctuations) ($p < 0.01$ for all bacteria, Fig. 2). The effect of temperature
197 fluctuations is similar to that of constant warming.

198 **Bacterial growth and T_{min}**

199 After 10 generations, the bacteria showed rapid adaptation. Square root growth curves of the bacteria
200 at each incubation temperature were obtained by fitting curves using the square root relationship, and
201 T_{min} was then fitted (extent of fitting the curves: P<0.001 for all the species, Fig. 3). A relatively
202 uniform trend was observed for all the species, i.e., T_{min} was elevated in all the experimental groups
203 compared to the control group. In this experiment, the increase in T_{min} varied among species in
204 response to a 15°C temperature increase (4.099°C, 0.536°C, 2.736°C, 4.255°C, 2.792°C and 2.259°C),
205 with an overall average increase of 2.78°C (n=6).

206 To verify the significance of the effect of temperature on bacterial growth, six bacterial species were
207 used as replicates to compare the effects of T_{min}. The bacterial T_{min} values obtained for the three
208 experimental incubation temperatures (20 ± 5°C, 20 ± 15°C and 35°C) were compared sequentially
209 with that of the control group (20°C). The results obtained showed significant increases in T_{min} in the
210 experimental groups compared to the control group ($p = 0.028$, $p = 0.014$, $p = 0.004$). Temperature
211 fluctuations and constant warming produced similar effects (Fig. 4).

212

213 # DISCUSSION

214 The results of our experiment confirmed that the responses of the respiration and growth of the
215 bacterial species following separation to thermal changes were similar to those observed from the
216 microbial community (Bárcenas-Moreno *et al*. 2009; Bradford *et al*. 2008). Individual bacterial species

217 also exhibited thermal adaptation phenomena. The decreasing respiration rates (Fig. 1) and Q_{10} values
218 (Fig. 2) of the six bacterial species indicated significant thermal adaptation, i.e., the bacteria that
219 underwent thermal adaptation had essentially unchanged R_{mass} values at relatively low temperatures
220 but decreased R_{mass} values at high temperatures compared to the bacteria in the control. Moreover, the
221 bacterial growth rate slowed after thermal adaptation and the T_{min} increased at lower test temperatures;
222 similarly, a slower growth rate was observed at higher test temperatures (compared to the control
223 group). By using individually cultured bacteria, our study provides, to our knowledge, the first
224 evidence of thermal adaptation by a single strain of bacteria, separate from abundant microbial
225 communities and related physiologies.

226 The thermal adaptations of the bacterial species were highly consistent, and the results were similar
227 to those from experiments on bacterial communities (Bradford *et al*. 2008; Li *et al*. 2021). Bacterial
228 communities typically exhibit redundant gene functions for multiple physiological phenomena in
229 response to environmental changes (Louca *et al*. 2018). While individual bacterial species lack such
230 genetic diversity, they also undergo thermal adaptation to increased temperatures, resulting in reduced
231 R_{mass} values at higher test temperatures. We believe such results to be related to changes in individual
232 bacterial protein conformations and the rapid adaptation of bacterial cell membranes (Wang *et al*. 2021).
233 Bacteria adapt to higher temperatures by reducing the maximum activity levels of proteins as well as
234 by increasing the half-saturation constant, similar to the changes in respiratory enzymes (Daniel *et al*.
235 2008; Hochachka & Somero 2002). Thermal adaptation of enzymes reduces the release of CO_2, thus
236 decreasing the peak respiration of bacteria. Moreover, the organic C use efficiency decreases, and the
237 biomass is limited, further increasing the T_{min} of the bacteria. The alteration of bacterial T_{min} also
238 originates in part from changes in the cell membrane due to the strong effect of temperature on the

239 fatty acid composition of the cell membrane (Hall *et al*. 2010). The membrane compositions of bacteria
240 can change to adapt to varying temperatures when the substrate is sufficient (Knothe & Dunn 2009).
241 Therefore, bacterial cell membranes must maintain a certain viscosity to sustain a stable physiology
242 and control membrane permeability to ions (Nichols & Deamer 1980). Stabilization of the cell
243 membrane allows bacteria to transport fewer protons (van de Vossenberg *et al*. 1999), leading to a
244 higher initial growth temperature at low test temperatures. Evidently, bacterial cell membranes can
245 adapt very quickly and strongly to temperature.

246 The response of bacterial T_{min} to changing temperature is very important and has great potential
247 to affect C release under continuously fluctuating environmental temperatures (Bååth 2018;
248 Nottingham *et al*. 2019). T_{min} can partly represent the strength of the temperature response and thermal
249 adaptation of bacteria (Davidson & Janssens 2006). T_{min} is usually considered the minimum
250 temperature for bacterial growth and respiration, which are normally expected to be similar (Li *et al*.
251 2021). As the most important decomposer in soil, the biomass and activity of bacteria may decrease
252 because of increased T_{min}, accompanied by a decrease in carbon use efficiency (Conant *et al*. 2011;
253 Hartley *et al*. 2007). Previous models have indicated that the T_{min} of soil bacteria increases by 0.2-
254 0.3°C per 1°C rise in environmental temperature (Bååth 2018; Li *et al*. 2021), similar to the results
255 observed for the 15°C warming group in this experiment (T_{min} increased by 2.765°C). In addition, it
256 has been experimentally proven that a significant thermal adaptation of the soil bacterial community
257 occurs after several years of warming and that the T_{min} of the bacteria does not change any further with
258 continued warming (Rinnan *et al*. 2009; Rousk *et al*. 2012). The results of these previous experiments
259 were similar to those of our experiment. Thus, we speculate that the increasing T_{min} of the soil bacterial
260 community may originate from these widely distributed dominant bacteria. Such changes may be due

261 to their individual adaptations (i.e., strong phenotypic plasticities) or the adaptation of bacterial
262 genotypes to warming (i.e., evolution), the demonstration of which requires more thorough
263 experiments.

264 The results indicate that the effect of temperature fluctuations on the bacteria was similar to that of
265 constant warming, and there was little difference due to the extent of the fluctuations. Although the
266 average temperature of the two incubation treatments with temperature fluctuations was the same as
267 that of the control group, the bacteria still responded to higher temperatures. It is evident that
268 temperature fluctuations within a narrow range also have a large impact on bacteria. Microbial
269 communities in environments with fluctuating temperatures, where changing temperature regimes
270 provide growth conditions for various species, promote species diversity, and maintain community
271 homeostasis (Upton *et al*. 1990). These findings also apply to individually cultured bacteria, which are
272 able to differentiate rapidly in environments with fluctuating conditions and sufficient nutrients to
273 avoid being eliminated from the environment, allowing the bacteria to develop specific tolerances in
274 response to temperature stress (Schimel *et al*. 2007; Zhao *et al*. 2021). Differentiated bacteria have
275 multiple mechanisms of physiological resilience and dormancy (Chesson 2000) that ensure the survival
276 of bacteria adapted to higher temperatures as a way to resist adverse environments. While previous
277 studies have largely explained how bacterial communities respond to temperature fluctuations (Jiang
278 & Morin 2007; Oliverio *et al*. 2017), our study provides the first evidence of adaptation by dominant
279 and representative soil bacteria to warming and temperature fluctuations through individual
280 cultivations. Such a physiological response of soil bacteria to temperature is expected to be
281 complementary to the predicted impact of warming on ecosystem C release.

282 The finding that bacteria have the ability to adapt to temperature enriches previous predictions of C

efflux from soil bacterial communities. Our experiment illustrates that thermal adaptation leads to a reduction in R_{mass} and growth of soil bacteria and that the adaptation may result from both warming and temperature fluctuations. When soils are exposed to high temperatures for long periods, bacterial growth becomes slower, respiration decreases, and the efficiency of soil organic C decomposition may decrease. Initially, bacterial responses to temperature changes arise from passive phenotypic plasticity and/or shifts in optimal trait expression. Sustained environmental fluctuations may cause species adaptations to revert to optimal bacterial traits (Stillman 2003). Microbes are responsible for a significant proportion of soil respiration; thus, the ability of bacteria to adapt to temperature will affect their capacity to cope with global warming.

Nevertheless, soil bacterial communities experience complex environmental changes, and culturing individual bacterial species cannot replicate the changes that occur in bacterial communities in situ. However, our study identified some of the mechanisms by which temperature effects decomposers in soil ecosystems and takes into account the effects of temperature fluctuations. There are new challenges for the future prediction of changes in soil respiration. In contrast to previous studies showing that soil microbial communities exhibit thermal adaptation for months after warming (Eliasson *et al*. 2005), our study shows that individual bacteria adapt quickly, within a few weeks or even days. Bacteria may adapt to temperatures in a shorter period of time than ecosystem-scale biomes, which also suggests that it is difficult to infer ecosystem community performance from the physiological phenomena of individual species. Future research is expected to focus on thermal adaptation in ecosystems at different scales or to explore the molecular mechanisms of temperature adaptation in depth in individual species. Our study also provides some evidence that individual soil bacterial species have the ability to adapt to varying temperatures, offering new ideas for future models

305 to predict soil respiration.

306

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CODE AVAILABILITY STATEMENT

The code used in this study is availability from the corresponding author upon reasonable request.

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477

Figure legends

Figure 1 Exponential curves of bacterial R_{mass} with increasing test temperatures. Respiration was measured after 10 generations and calculated as R_{mass} at four test temperatures (5°C, 15°C, 25°C and 35°C). The incubation temperatures of the bacteria were 20°C (blue), 20 ± 5°C (green), 20 ± 15°C (yellow) and 35°C (red). The trends were observed by using exponential model fitting curves.

Figure 2 T-tests were performed for the respiratory Q_{10} of the control and experimental groups. After calculating the Q_{10} of all the bacteria at all the test temperatures (5–35°C), t-tests were performed in R. The incubation temperatures of the bacteria were 20°C (red), 20 ± 5°C (green), 20 ± 15°C (blue) and 35°C (purple). The numbers above the lines are significant P values.

Figure 3 A square root model was used to fit the curves of the bacterial growth rate with respect to temperature. The test temperatures were set to 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. The incubation temperatures for bacterial growth were 20°C (blue), 20 ± 5°C (green), 20 ± 15°C (yellow) and 35°C (red).

Figure 4 The trends of T_{min}, a bacterial growth index. The data obtained for the experimental group were compared with those for the control group with a paired t-test. The three red boxes represent the results of the control group (20°C), and the three purple boxes represent the experimental groups, with incubation temperatures of 20 ± 5°C, 20 ± 15°C and 35°C.

Figure 1.

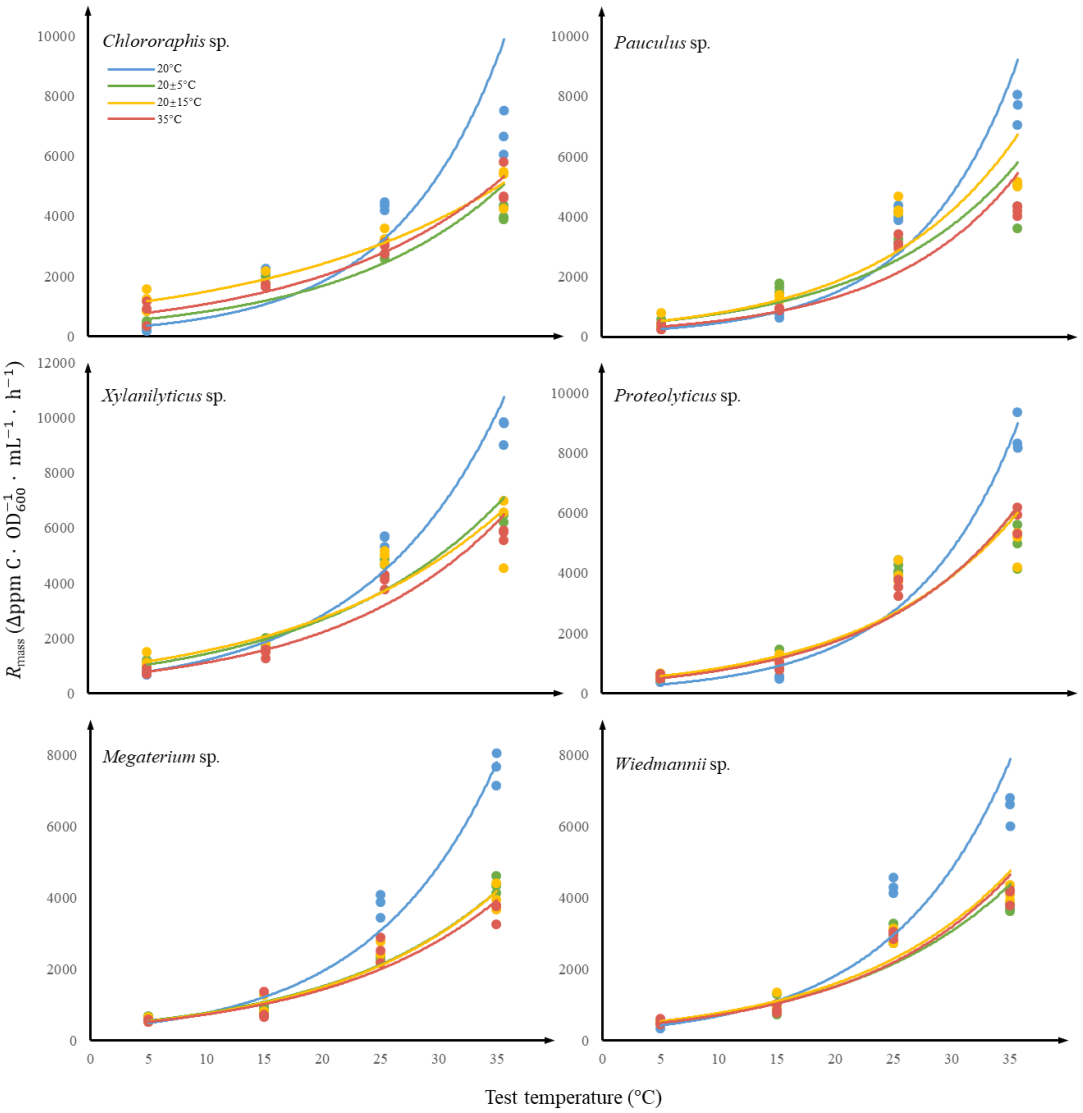
Chlororaphis sp.
20°C
20±5°C
20±15°C
35°C
Pauculus sp.
Xylanilyticus sp.
Proteolyticus sp.
Megaterium sp.
Wiedmannii sp.
R_{mass} (Δppm C · OD_{600}^{-1} · mL^{-1} · h^{-1})
Test temperature (°C)

Figure 2.

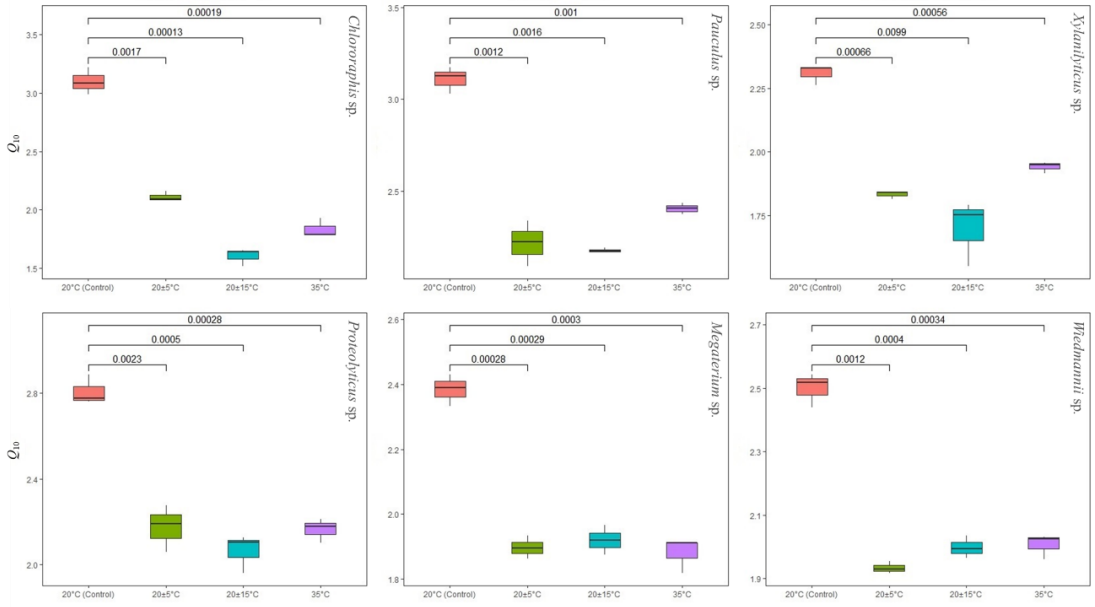
0.00019
0.00013
0.0017
Chlororaphis sp.
0.001
0.0016
0.0012
Pauculus sp.
0.00056
0.0099
0.00066
Xylanilyticus sp.
0.00028
0.0005
0.0023
Proteolyticus sp.
0.0003
0.00029
0.00028
Megaterium sp.
0.00034
0.0004
0.0012
Weadmanni sp.
Q_{10}
20°C (Control)
20±5°C
20±15°C
35°C

Figure 3.

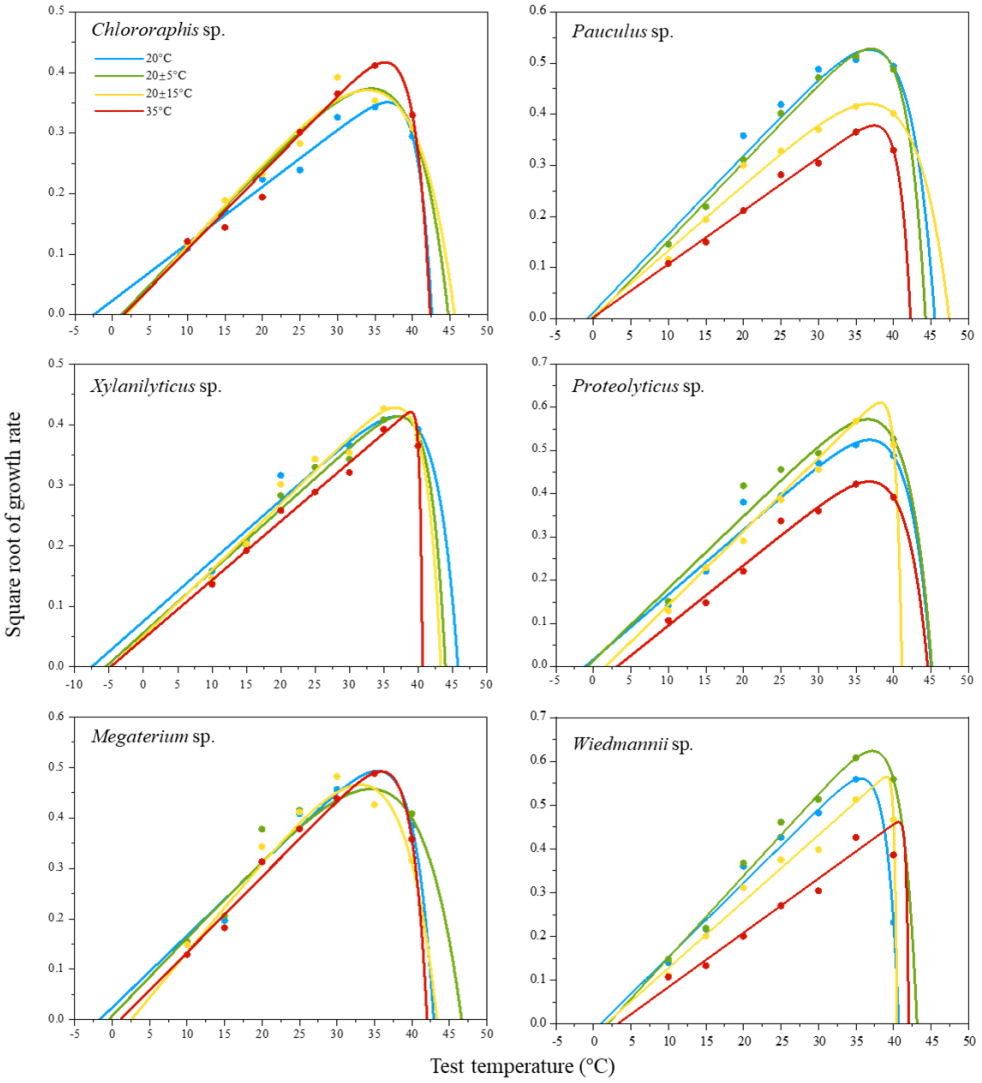
Chlororaphis sp.
20°C
20±5°C
20±15°C
35°C
Pauculus sp.
Xylanilyticus sp.
Proteolyticus sp.
Megaterium sp.
Wiedmannii sp.
Square root of growth rate
Test temperature (°C)

Figure 4.